STABLE PHARMACEUTICAL COMPOSITION OF RECEPTOR AGONIST, AND PREPARATION METHOD AND USE THEREOF

Technical Field

The present invention relates to the field of pharmaceutical preparations. Specifically, the present invention relates to a stable pharmaceutical composition of a GLP-1/GIP receptor agonist, a preparation method therefor, and use thereof.

Background Art

Over the past 50 years, the prevalence of diabetes mellitus has continued to increase worldwide, and the number of affected people is expected to reach 700 million by 2050. The prevalence of diabetes mellitus in China is 11.2%, with about 130 million of affected people. China is currently the country with the largest number of diabetic patients, which has become one of the major public health problems, bringing huge physical, psychological and economic burdens to patients, families and society. Type 2 Diabetes Mellitus (T2DM) accounts for more than 90% of the diabetic population and is primarily pathophysiologically characterized by a decrease in the ability of insulin to regulate glucose metabolism (i.e., insulin resistance) with a concomitant decrease in insulin secretion caused by pancreatic β cell dysfunction.

T2DM medications fall into three main categories, oral hypoglycemic drugs, GLP-1 receptor agonists (GLP-1 RAs) and insulin/insulin analogues. Oral hypoglycemic drugs mainly include insulin secretagogues (sulfonylureas, non-sulfonylureas), insulin sensitizers (metformin, thiazolidinediones), etc. Metformin is the first choice for clinical treatment, which has a strong blood glucose lowering effect, a lower risk of hypoglycemia, and a body weight reducing advantage. If blood glucose control is not good, double or triple therapy is performed, in combination with insulin secretagogues, dipeptidyl peptidase-4 (DPP-4) inhibitors, GLP-1 RAs, etc. For obese T2DM patients, metabolic surgery can be performed to reduce body weight while alleviating or even reversing the diabetic condition. After metabolic surgery, intestinal hormones change, and incretin secretion effect is enhanced, to achieve metabolic effects such as body weight reduction and blood glucose decrease. Incretin secretion effect is one of the main causes of increased insulin secretion after eating in healthy populations, wherein the two intestinal hormones that play an important role are GLP-1 and GIP, respectively.

After eating, T2DM patients exhibit reduced secretion of GLP-1, which binds to a receptor, and then exhibits an insulin secretion stimulating effect similar to that in healthy populations. GLP-1 in a human body has a very short half-life because it can be rapidly degraded by DPP-4. Regarding the drug development for this mechanism, human endogenous GLP-1 is restructured to function with a longer half-life in the human body. In recent decades, the development of GLP-1 RAs has progressed rapidly, and in addition to their obvious blood glucose lowering effects, they also have an obvious body weight reducing advantage. Compared with placebo groups, they can also obviously reduce the risk of major cardiovascular events and all-cause mortality. In addition to the treatment of diabetes mellitus, they have also been developed for the treatment of overweight or obese non-diabetic patients. Duraglutide and semaglutide among GLP1-RAs have significant effects, wherein duraglutide was marketed in China in 2019, and has obviously better blood glucose lowering and body weight reducing effects than liraglutide. Semaglutide has better blood glucose lowering and body weight reducing effects than dulaglutide, and similar safety to dulaglutide.

Long-term treatment with high concentrations of GLP-1 RAs can maintain good blood glucose lowering and body weight reducing effects, but severe gastrointestinal side effects such as nausea and vomiting limit the use of the maximum dose of GLP-1 Ras, thereby failing to achieve their potential maximum efficacy, so that there still remains a large gap between the efficacy of GLP-1 RAs on T2DM and that of gastrointestinal metabolic surgery. At present, a multi-target drug development strategy based on the mechanism of action of GLP-1 RAs is proposed, wherein another GIP with incretin effect is an important research target. The blood glucose lowering effect of GIP is severely impaired at high blood glucose concentrations in T2DM patients, but its insulin secretion stimulating effect can be quickly restored when blood glucose concentrations return to normal levels. Moreover, GIP can regulate abnormal fat metabolism, and reduce abnormal fat accumulation between tissues, thus improving insulin sensitivity, while acting synergistically with GLP-1 in the central nervous system to reduce food intake. GIP can further reduce gastrointestinal reactions such as nausea and vomiting caused by an antineoplastic drug cisplatin, possibly further enhances efficacy by increasing the tolerance of GLP-1 RAs.

The active ingredient of the present invention is a new generation of GIP/GLP-1 dual-receptor agonist developed by our company, having a very strong agonistic activity on both the GIP receptor and the GLP-1 receptor. With the dual-receptor agonist activity, the dual-receptor agonist is intended for the treatment of type 2 diabetes mellitus (T2DM). The pharmacodynamic experiment results in animals show that: after single administration, the present invention can significantly reduce the

random blood glucose in db/db diabetic mice, stimulate insulin secretion and reduce food intake, and has a certain body weight reducing effect, and the pharmaceutical composition of the present invention has a better therapeutic effect than semaglutide at an equivalent dose. After long-term continuous administration once every 3 days, the pharmaceutical composition of the present invention can dose-dependently reduce the blood glucose, body weight, and liver weight of db/db diabetic mice; in the continuous administration test, the pharmaceutical composition of the present invention can dose-dependently reduce the body weight of DIO mice, reduce the food intake and liver weight, and further obviously reduce the triglyceride, low density lipoprotein cholesterol and total cholesterol levels in the plasma of DIO (diet-induced obesity) mice, and improve the blood lipid metabolism disorder in DIO mice; and the pharmaceutical composition of the present invention has better blood lipid lowering and body weight reducing effects than those of semaglutide at an equivalent dose. Thus, it can be seen that the active ingredient of the present invention has a good blood glucose lowering effect, can play a role in reducing blood lipid and body weight, and has a good development prospect.

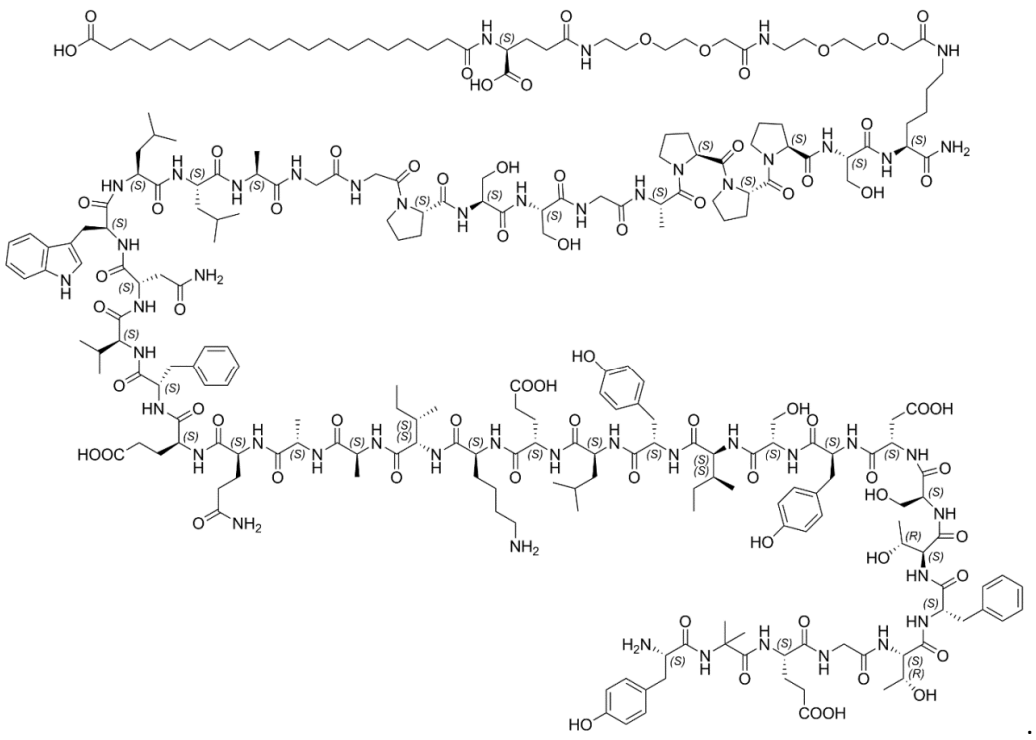
The active ingredient in the present invention is a polypeptide, which is a compound formed by linking a plurality of amino acids together via peptide bonds. The stressing test results of the bulk drug show occurrence of obvious degradation under conditions of an acid, an alkali, oxidation, or a high temperature; and the influencing factor test results of the bulk drug show polymer growth of the bulk drug under conditions of a high temperature or illumination. The special properties of the active ingredient result in poor stability of the active ingredient. In this case, it remains a great challenge for researchers to obtain a stable, high-quality, industrially producible pharmaceutical composition of a GLP-1/GIP receptor agonist.

Summary of the Invention

An object of the present invention is to provide a stable pharmaceutical composition of a GIP/GLP-1 dual-receptor agonist.

The object of the present invention is achieved through the following technical solutions:

the pharmaceutical composition of the present invention comprises an active ingredient, a buffer solution, an osmotic pressure regulator and a pH regulator, and the active ingredient of the present invention has a structure of:



The amino acid sequence of the structure is as follows:

L-tyrosyl-isobutyryl-L-glutamyl-glycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-aspartyl-L-tyrosyl-L-seryl-L-isoleucyl-L-tyrosyl-L-leucyl-L-glutamyl-L-lysyl-L-isoleucyl-L-alanyl-L-alanyl-L-glutaminyl-L-glutamyl-L-phenylalanyl-L-valyl-L-asparaginyl-L-tryptophanyl-L-leucyl-L-leucyl-L-alanyl-glycyl-glycyl-L-prolyl-L-seryl-L-seryl-glycyl-L-alanyl-L-prolyl-L-prolyl-L-prolyl-L-seryl-{N6-[(22S)-10,19,24-trioxo-3,6,12,15-tetraoxa-9,18,23-triaza-22,42-dicarboxy-tetradodecanoyl]}-L-lysinamide.

Preferably, the pharmaceutical composition of the GIP/GLP-1 dual-receptor agonist is an injection, with the active ingredient at a concentration selected from 0.5 mg/mL-40 mg/mL, preferably 1 mg/mL-30 mg/mL, and more preferably 1 mg/mL, 2 mg/mL, 5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL, or 30 mg/mL.

Preferably, the injection comprises 0.05%-4.0% (W/V) of the active ingredient, preferably 0.1%-4.0% (W/V), and more preferably 0.1%- 3.0% (W/V).

In the present invention, the unit of W/V is g/mL.

Preferably, the buffer solution is selected from a phosphate buffer solution, an acetate buffer solution, a citrate buffer solution, a carbonate buffer solution, a tartrate buffer solution, a Tris

buffer solution, and a histidine salt, preferably a citrate buffer solution or a phosphate buffer solution, and more preferably disodium hydrogen phosphate.

Preferably, the injection comprises 0.05%3.0% (W/V) of the buffer solute, preferably 0.05%-2.0% (W/V), and more preferably 0.05%- 1.0% (W/V).

Preferably, the osmotic pressure regulator is selected from one or more of mannitol, lactose, sucrose, propylene glycol, and glycerol, preferably propylene glycol or mannitol.

Preferably, the injection comprises 0.05%-5.0% (W/V) of the osmotic pressure regulator, preferably 1.0%- 3.0% (W/V), and more preferably 1.0%- 2.0% (W/V).

Preferably, the pH regulator is selected from one or more of hydrochloric acid or sodium hydroxide.

Preferably, the injection comprises 0.1%- 3.0% (W/V) of the active ingredient, 0.05%- 3.0% (W/V) of the buffer solute, 0.5%- 5.0% (W/V) of the osmotic pressure regulator, and an appropriate amount of the pH regulator.

Preferably, the injection comprises 0.1%- 3.0% (W/V) of the active ingredient, 0.05%- 2.0% (W/V) of the buffer solute, 1.0%- 3.0% (W/V) of the osmotic pressure regulator, and an appropriate amount of the pH regulator.

Preferably, the injection comprises 0.1%- 3.0% (W/V) of the active ingredient, 0.05%- 1.0% (W/V) of the buffer solute, 1.0%- 2.0% (W/V) of the osmotic pressure regulator, and an appropriate amount of the pH regulator.

Preferably, the injection comprises 0.1%- 3.0% (W/V) of the active ingredient, 0.05%- 0.1% (W/V) of the buffer solute, 1.0%- 2.0% (W/V) of the osmotic pressure regulator, and an appropriate amount of the pH regulator.

Preferably, the injection comprises 0.1%- 3.0% (W/V) of the active ingredient, 0.05%- 1.0% (W/V) of disodium hydrogen phosphate and/or sodium hydroxide, 1.0%-2.0% (W/V) of propylene glycol, and an appropriate amount of the pH regulator.

Optionally, the injection further comprises a preservative selected from m-cresol, phenol, phenylcarbinol, phenethyl alcohol, parahydroxybenzoate, hydroxybenzoate, benzyl alcohol, chlorobutanol, phenoxyethanol, methyl paraben, etc., preferably m-cresol, phenol, or trichloro-tert-butanol.

Optionally, the injection comprises 0.01%- 3% (W/V) of the preservative, preferably comprises 0.05%- 1.5% (W/V) of the preservative, more preferably comprises 0.1%- 1% (W/V) of the preservative, and further preferably comprises 0.1%- 0.5% (W/V) of the preservative.

Preferably, the injection comprises 0.1%- 3.0% (W/V) of the active ingredient, 0.05%- 1.0% (W/V) of disodium hydrogen phosphate and/or sodium hydroxide, 1.0%-2.0% (W/V) of propylene glycol, 0.05%-1.5% (W/V) of the preservative, and an appropriate amount of the pH regulator.

Preferably, the injection comprises 0.1%-3.0% (W/V) of the active ingredient, 0.05%-1.0% (W/V) of disodium hydrogen phosphate and/or sodium hydroxide, 1.0%-2.0% (W/V) of propylene glycol, 0.1%-1% (W/V) of the preservative, and an appropriate amount of pH regulator.

Preferably, the injection comprises 0.1% to 3.0% (W/V) of the active ingredient, 0.05% to 1.0% (W/V) of disodium hydrogen phosphate and/or sodium hydroxide, 1.0% to 2.0% (W/V) of propylene glycol, 0.1% to 0.5% (W/V) of the preservative, and an appropriate amount of pH regulator.

Preferably, a weight ratio of the active ingredient to the buffer solute is 1 : 0.01-10, preferably 1 : 0.02-1, and more preferably 1 : 0.02-0.5.

Preferably, a weight ratio of the active ingredient to the osmotic pressure regulator is 1 : 0.1-20, preferably 1 : 0.4-10.

Preferably, a ratio of total amount of disodium hydrogen phosphate to total amount of sodium hydroxide in the buffer solution is 1 : 0-3.0, preferably 1 : 0.10-2.5, more preferably 1 : 0.13-1.5, and further preferably 1 : 0.13-1.2.

Preferably, a weight ratio of the buffer solute to the osmotic pressure regulator is 1 : 5-80, preferably 1 : 5-60, more preferably 1 : 15-40, and further preferably 1 : 15-25.

Preferably, the pharmaceutical composition is in a pH range of 6.5-9.0, preferably 7.0-8.5, and more preferably 7.0-8.0.

Preferably, dosage volume of the pharmaceutical composition is 0.5 mL-1 mL.

Another object of the present invention is to provide a method for preparing the pharmaceutical composition, wherein the buffer solute and the osmotic pressure regulator are dissolved in water for injection, is dissolved in the above drug solution by stirring, the pH regulator is added, and the mixture is made up to volume, filtered, and subpackaged.

Preferably, the water for injection is cooled to ≤ 25°C.

Preferably, the resulting pharmaceutical composition is aseptically filtered, subpackaged, and encapsulated.

Preferably, the filling process is completed under nitrogen protection.

Preferably, the pharmaceutical composition is subpackaged into prefilled syringes.

Preferably, the pharmaceutical composition is subpackaged into cartridge bottles.

Preferably, the pharmaceutical composition is subpackaged into vials.

Another object of the present invention is to provide use of the pharmaceutical composition for the manufacture of a medicament for treating non-insulin-dependent diabetes mellitus, insulin-dependent diabetes mellitus, obesity, insulin resistance, or blood lipid metabolism disorder, wherein preferably, the non-insulin-dependent diabetes mellitus is type II diabetes mellitus.

The pharmaceutical composition of the present invention can be simultaneously, separately, or successively used in combination with one or more reagents selected from metformin, thiazolidinediones, sulfonylureas, dipeptidyl peptidase inhibitors and sodium glucose transporters.

Drugs are different from other chemical products, and even a very tiny amount of impurities may pose risks to the safety, efficacy, quality controllability, etc. of clinical medication. In the present invention, a pharmaceutical composition having good stability can be obtained by controlling the ingredients and proportions of the ingredients in the formula without adding other auxiliary materials, wherein the content of related substances is effectively controlled and the content of particular impurities is obviously reduced so as to greatly reduce the toxicity of the drug, and the content of particular impurities increases slowly after prolonged storage of the drug, so as to improve the safety of the drug. Further, stability of the preparation is improved by filling under nitrogen protection.

Detailed Description

It should be understood that those skilled in the art can make various modifications and improvements to the present invention based on the content disclosed herein without departing from the spirit and scope of the present invention. The various modifications and improvements should all be encompassed within the scope of protection as defined by the claims of the present application.

Furthermore, it should be understood that examples provided herein are only for the purpose of illustrating the present invention and should not be construed as a limitation on the present invention.

Example I

Ingredient	Use amount
Active ingredient	0.2 g, 1.0 g, 2.0 g, 3.0 g, 4.0 g
Disodium hydrogen phosphate[1]	0.071 g
Propylene glycol	1.5 g
Sodium hydroxide[2]	0.01 g
Sodium hydroxide	Q.S.
Hydrochloric acid	Q.S.
Water for injection	Balanced to 100 mL

Note 1: 5 mM disodium hydrogen phosphate solution was used.

Note 2: 100 g/L sodium hydroxide solution was used.

The water for injection was cooled to ≤ 25°C and added for later use. Disodium hydrogen phosphate (0.071 g) was weighed according to the formula, dissolved in 90 mL of the water for injection, and stirred for dissolution; 0.1 mL of 100 g/L sodium hydroxide solution was then added and fully stirred (0.2 g of the active ingredient was not added); propylene glycol (1.5 g) was weighed according to the formula and fully stirred; the active ingredient of the present invention was weighed according to the formula and slowly stirred for dissolution, the drug solution was adjusted with a sodium hydroxide or hydrochloric acid solution to a pH of 7.5-8.0; and the above drug solution was made up to 100mL with the water for injection, finally the drug solution was filtered through a 0.22 μm microporous filter membrane, the filtered drug solution was subpackaged into prefilled syringe bottles, the bottles were filled with nitrogen, and stoppers were pressed into the bottles. The samples were inspected for stability under conditions of 25°C ± 2°C/60% RH ± 5% RH and 40°C, respectively, the inspection indicators including appearance and related substances, with the results as shown in the table below.

Active ingredient	Conditions	Time	Appearance	Total impurity (%)
0.2 g	25°C ± 2°C/ 60% RH ± 5% RH	0 d	Colorless clear liquid	2.5
		6 M	Colorless clear liquid	7.0
1.0 g		0 d	Colorless clear liquid	2.6
		6 M	Colorless clear liquid	7.3
2.0 g		0 d	Colorless clear liquid	2.6

		6 M	Colorless clear liquid	7.3
3.0 g		0 d	Colorless clear liquid	2.5
		6 M	Colorless clear liquid	7.2
4.0 g		0 d	Colorless clear liquid	2.6
		6 M	Colorless clear liquid	7.3
0.2 g	40°C	0 d	Colorless clear liquid	2.5
		14 d	Colorless clear liquid	4.6
1.0 g		0 d	Colorless clear liquid	2.6
		14 d	Colorless clear liquid	5.0
2.0 g		0 d	Colorless clear liquid	2.6
		14 d	Colorless clear liquid	5.0
3.0 g		0 d	Colorless clear liquid	2.4
		14 d	Colorless clear liquid	5.1
4.0 g		0 d	Colorless clear liquid	2.5
		14 d	Colorless clear liquid	5.3

Example II

Ingredient	Use amount
Active ingredient	2.0 g
Disodium hydrogen phosphate[1]	0.071 g
Sodium hydroxide[2]	0.01 g
Propylene glycol	1.5 g
Sodium hydroxide	Q.S.
Hydrochloric acid	Q.S.
Water for injection	Balanced to 100 mL

Note 1: 5 mM disodium hydrogen phosphate solution was used.

Note 2: 100 g/L sodium hydroxide solution was used.

The water for injection was cooled to ≤ 25°C and added for later use. Disodium hydrogen phosphate (0.071 g) was weighed according to the formula, dissolved in 90 mL of the water for injection, and stirred for dissolution; 0.1 mL of 100 g/L sodium hydroxide solution was then added and fully stirred; propylene glycol (1.5 g) was weighed according to the formula and fully stirred; the active ingredient (2.0 g) of the present invention was weighed according to the formula and slowly stirred for dissolution, the drug solution was adjusted with a sodium hydroxide or hydrochloric acid solution to a pH of 6.5, 7.0, 7.5,

7.8, 8.0, 8.5 respectively; and each of the above drug solutions was made up to 100 mL with the water for injection, finally the drug solution was filtered through a 0.22 μm microporous filter membrane, the filtered drug solution was subpackaged into prefilled syringe bottles, the bottles were filled with nitrogen, and stoppers were pressed into the bottles. The samples were inspected for stability under conditions of 25°C ± 2°C/60% RH ± 5% RH, and the inspection indicators including appearance and related substances, with the results as shown in the table below.

pH	Conditions	Time	Appearance	Total impurity (%)
6.5	25°C ± 2°C/ 60% RH ± 5% RH	0 d	Colorless clear liquid	2.2
		1 M	Transparent gelatinous precipitation	/
7.0		0 d	Colorless clear liquid	2.2
		1 M	Colorless clear liquid	2.5
7.5		0 d	Colorless clear liquid	2.2
		1 M	Colorless clear liquid	2.9
7.8		0 d	Colorless clear liquid	2.2
		1 M	Colorless clear liquid	3.0
8.0		0 d	Colorless clear liquid	2.1
		1 M	Colorless clear liquid	3.1
8.5		0 d	Colorless clear liquid	2.1
		1 M	Colorless clear liquid	3.6

Example III

Ingredient	Use amount
Active ingredient	2.0 g
Disodium hydrogen phosphate[1]	0.071 g
Sodium hydroxide[2]	Amount in the formula
Propylene glycol	1.5 g
Water for injection	Balance to 90 mL

Note 1: 5 mM disodium hydrogen phosphate solution was used.

Note 2: 100 g/L sodium hydroxide solution was used.

The water for injection was cooled to ≤ 25°C and added for later use. Disodium hydrogen phosphate (0.071 g) was weighed according to the formula, dissolved in 90 mL of the water for injection, and stirred for dissolution; 100 g/L sodium hydroxide solution was then added according to the formula and fully stirred; propylene glycol (1.5 g) was weighed according to the formula and fully stirred; the pH value of the solution was determined, the active ingredient (2.0 g) of the present invention was weighed according to the formula, and slowly added, the mixture was stirred for 45 min, then the dissolution phenomenon of the active ingredient was observed, the pH value of the solution was determined, the samples were inspected for stability under conditions of 25°C ± 2°C/60% RH ± 5% RH, 5°C ± 3°C, 40°C and illumination, and the impurity components of the samples were subjected to analysis study to inspect the effects of different buffer solution concentrations on the impurity components, thus providing a research idea for selection of the pharmaceutical composition, with the study results as shown in the table below.

Addition amount of 100 g/L sodium hydroxide	pH before addition of active ingredient	pH after addition of active ingredient	Dissolution phenomenon	Inspection conditions	Time	Related substances (%)		
						RRT1.06	RRT1.14	Total impurity
0.8 mL	12.0	6.8	Colorless clear liquid, completely dissolved	0 d		0.20	0.02	2.1
				25°C ± 2°C/ 60% RH ± 5% RH	1 M	0.24	0.28	4.4
				5°C ± 3°C	1 M	0.25	0.30	4.5
				40°C	1 M	0.31	0.58	6.7
				Illumination	15 d	0.30	0.70	7.5
0.3 mL	11.7	6.6	Colorless clear liquid, completely dissolved	0 d		0.18	0.02	2.0
				25°C ± 2°C/ 60% RH ± 5% RH	1 M	0.23	0.25	4.3
				5°C ± 3°C	1 M	0.22	0.35	4.3
				40°C	1 M	0.29	0.59	6.6
				Illumination	15 d	0.33	0.65	7.3
0.1 mL	10.8	5.7	Colorless clear liquid,	0 d		0.20	Not detected	2.0

			completely dissolved	25°C ± 2°C/ 60% RH ± 5% RH	1 M	0.22	0.25	4.3
				5°C ± 3°C	1 M	0.22	0.35	4.2
				40°C	1 M	0.28	0.55	6.5
				Illumination	15 d	0.38	0.65	7.2
0.08 mL	10.3	/	Off-white solution, incompletely dissolved	0 d		0.23	0.45	9.0

Example IV

Ingredient	Use amount
Active ingredient	2.0 g
Disodium hydrogen phosphate	Amount in the formula
Sodium hydroxide[1]	0.01 g
Propylene glycol	1.5 g
Sodium hydroxide	Q.S.
Hydrochloric acid	Q.S.
Water for injection	Balance to 100 mL

Note 1: 100 g/L sodium hydroxide solution was used.

The water for injection was cooled to ≤ 25°C and added for later use. Disodium hydrogen phosphate was weighed according to the formula, dissolved in 90 mL of the water for injection, and stirred for dissolution; 0.1 mL of 100 g/L sodium hydroxide solution was then added and fully stirred; propylene glycol (1.5 g) was weighed according to the formula and fully stirred; the active ingredient (2.0 g) of the present invention was weighed according to the formula and slowly stirred for dissolution, the drug solution was adjusted with a sodium hydroxide or hydrochloric acid solution to a pH of 7.5-8.0 respectively; each of the above drug solutions was made up to 100 mL with the water for injection, finally the drug solution was filtered through a 0.22 μm microporous filter membrane, the filtered drug solution was subpackaged into prefilled injection bottles, the bottles were filled with nitrogen, and stoppers were pressed into the bottles. The samples were inspected for stability under conditions of 25°C ± 2°C/60% RH

± 5% RH or 40°C, the inspection indicators including appearance and related substances, with the results as shown in the table below.

Disodium hydrogen phosphate concentration	Conditions	Time	Appearance	Total impurity (%)
4 mM	25°C ± 2°C/ 60% RH ± 5% RH	0 d	Colorless clear liquid	2.7
		1 M	Colorless clear liquid	3.8
5 mM		0 d	Colorless clear liquid	2.7
		1 M	Colorless clear liquid	3.5
6 mM		0 d	Colorless clear liquid	2.7
		1 M	Colorless clear liquid	3.6
7 mM		0 d	Colorless clear liquid	2.0
		1 M	Colorless clear liquid	3.6
8 mM		0 d	Colorless clear liquid	2.1
		1 M	Colorless clear liquid	3.6
11 mM		0 d	Colorless clear liquid	2.1
		1 M	Colorless clear liquid	3.7
4 mM	40°C	0 d	Colorless clear liquid	2.7
		14 d	Colorless clear liquid	4.1
5 mM		0 d	Colorless clear liquid	2.7
		14 d	Colorless clear liquid	3.8
6 mM		0 d	Colorless clear liquid	2.7
		14 d	Colorless clear liquid	4.1
7 mM		0 d	Colorless clear liquid	2.0
		1 M	Colorless clear liquid	4.4
8 mM		0 d	Colorless clear liquid	2.1
		1 M	Colorless clear liquid	4.3
11 mM		0 d	Colorless clear liquid	2.1
		14 d	Colorless clear liquid	4.5

Example V

Ingredient	Use amount
Active ingredient	2.0 g
Disodium hydrogen phosphate[1]	0.071 g
Sodium hydroxide[2]	0.01 g
Osmotic pressure regulator	Amount in the formula
Sodium hydroxide	Q.S.
Hydrochloric acid	Q.S.
Water for injection	Balance to 100 mL

Note 1: 5 mM disodium hydrogen phosphate solution was used.

Note 2: 100 g/L sodium hydroxide solution was used.

The water for injection was cooled to ≤ 25°C and added for later use. Disodium hydrogen phosphate (0.071 g) was weighed according to the formula, and dissolved in 90 mL of the water for injection, and stirred for dissolution; 0.1 mL of 100 g/L sodium hydroxide solution was then added and fully stirred; an osmotic pressure regulator was weighed according to the formula and fully stirred; the active ingredient (2.0 g) of the present invention was weighed according to the formula and slowly stirred for dissolution, and the drug solution was adjusted with a sodium hydroxide or hydrochloric acid solution to a pH of 7.5-8.0 respectively; and the above drug solution was made up to 100mL with the water for injection, finally the drug solution was filtered through a 0.22 μm microporous filter membrane, the filtered drug solution was subpackaged into prefilled injection bottles, the bottles were filled with nitrogen, and stoppers were pressed into the bottles. The samples were inspected for stability under conditions of 25°C ± 2°C/60% RH ± 5% RH, the inspection indicators including appearance and related substances, with the results as shown in the table below.

Osmotic pressure regulator	Conditions	Time	Appearance	Total impurity (%)
Propylene glycol 1.5 g	25°C ± 2°C/ 60% RH ± 5% RH	0 d	Colorless clear liquid	2.6
		1 M	Colorless clear liquid	3.4
Mannitol 4.64 g		0 d	Colorless clear liquid	2.7
		1 M	Colorless clear liquid	3.7
Glycerin 3.6 g		0 d	Colorless clear liquid	4.2
		1 M	Colorless clear liquid	5.8

Lactose 9.5 g		0 d	Colorless clear liquid	4.4
		1 M	Colorless clear liquid	5.6
Sucrose 9.0 g		0 d	Colorless clear liquid	4.2
		1 M	Colorless clear liquid	5.8

Example VI

Component	Use amount
Active ingredient	2.0 g
Disodium hydrogen phosphate	Amount in the formula
Sodium hydroxide[1]	0.01 g
Propylene glycol	Amount in the formula
Sodium hydroxide	Q.S.
Hydrochloric acid	Q.S.
Water for injection	Balance to 100 mL

Note 1: 100 g/L sodium hydroxide solution was used.

The water for injection was cooled to ≤ 25°C and added for later use. Disodium hydrogen phosphate was weighed according to the formula, dissolved in 90 mL of the water for injection, and stirred for dissolution; 0.1 mL of 100 g/L sodium hydroxide solution was then added and fully stirred; propylene glycol was weighed according to the formula and fully stirred; the active ingredient (2.0 g) of the present invention was weighed according to the formula and slowly stirred for dissolution, and the drug solution was adjusted with a sodium hydroxide or hydrochloric acid solution to a pH of 7.5-8.0 respectively; and the above drug solution was made up to 100mL with the water for injection, finally the drug solution through filtered with a 0.22 μm microporous filter membrane, and the filtered drug solution was subpackaged into prefilled injection into which stoppers were pressed into the bottles. The samples were inspected for stability under conditions of 25°C ± 2°C/60% RH ± 5% RH, 5°C ± 3°C, 40°C, illumination and low temperature cycling, the inspection indicators including appearance and related substances, with the results are as shown in the table below.

Disodium hydrogen phosphate: propylene glycol (W/V%)	Conditions	Appearance	Total impurity (%)
1 : 15	0 d	Colorless clear liquid	2.1

Disodium hydrogen phosphate: propylene glycol (W/V%)	Conditions		Appearance	Total impurity (%)
	25°C ± 2°C/60% RH ± 5% RH	1 M	Colorless clear liquid	4.4
	5°C ± 3°C	1 M	Colorless clear liquid	4.2
	40°C	7 d	Colorless clear liquid	4.5
	Illumination	15 d	Colorless clear liquid	5.4
	Low temperature cycling	2 times	Colorless clear liquid	4.3
1 : 20	0 d		Colorless clear liquid	2.0
	25°C ± 2°C/60% RH ± 5% RH	1 M	Colorless clear liquid	4.3
	5°C ± 3°C	1 M	Colorless clear liquid	4.2
	40°C	7 d	Colorless clear liquid	4.7
	Illumination	15 d	Colorless clear liquid	5.5
	Low temperature cycling	3 times	Colorless clear liquid	4.5
1 : 25	0 d		Colorless clear liquid	2.0
	25°C ± 2°C/60% RH ± 5% RH	1 M	Colorless clear liquid	4.0
	5°C ± 3°C	1 M	Colorless clear liquid	4.2
	40°C	7 d	Colorless clear liquid	4.8
	Illumination	15 d	Colorless clear liquid	5.6
	Low temperature cycling	3 times	Colorless clear liquid	4.6
1 : 40	0 d		Colorless clear liquid	2.0
	25°C ± 2°C/60% RH ± 5% RH	1 M	Colorless clear liquid	4.0
	5°C ± 3°C	1 M	Colorless clear liquid	4.4
	40°C	7 d	Colorless clear liquid	4.7

Disodium hydrogen phosphate: propylene glycol (W/V%)	Conditions		Appearance	Total impurity (%)
	Illumination	15 d	Colorless clear liquid	5.5
	Low temperature cycling	3 times	Colorless clear liquid	4.7
1 : 80	0 d		Colorless clear liquid	
	25°C ± 2°C/60% RH ± 5% RH	1 M	White gelatinous precipitation	Not detected
	5°C ± 3°C	1 M	White gelatinous precipitation	Not detected
	40°C	7 d	Colorless clear liquid	5.9
	Illumination	15 d	Colorless clear liquid	7.0
	Low temperature cycling	3 times	Colorless clear liquid	5.8

Experimental Example I: Hemolysis test

1. Test substance:

Test sample: the injection prepared in Example I (with 2.0 g of the active ingredient);

1.2 Vehicle: sodium chloride injection.

2. Experimental method:

Different volumes (0.5-0.1 mL) of the test sample and different volumes (2.0-2.4 mL) of the sodium chloride injection were respectively added to 2.5 mL of 2% rabbit erythrocyte suspension present in glass test tubes, and the sodium chloride injection and sterilized water for injection were used as a negative control and a positive control, respectively, with a total volume of 5.0 mL per test tube. Each test tube was incubated in an electrothermostat (set at a temperature of 37°C) for 3 h, to observe the lysis and aggregation of erythrocytes.

As can be seen from the test tube containing 0.5-0.1 mL of the test sample and the negative control test tube, the erythrocytes were deposited at the bottom of the tube, the supernatant was colorless and clear, and the erythrocytes at the bottom of the tube, after being shaken, were fully

dispersed without hemolysis and without aggregation; and as can be seen from the positive control tube containing sterilized water for injection, some erythrocytes were deposited at the bottom of the tube, the supernatant was clear and red, and the erythrocytes at the bottom of the tube, after being shaken, were fully dispersed with partial hemolysis and without aggregation.

Under the experimental conditions, in the experimental system, 0.1-0.5 mL of the injectable pharmaceutical composition of the present invention at a labeled concentration of 20 mg/mL neither exhibited rabbit erythrocyte hemolysis, nor resulted in erythrocyte aggregation.

Experimental Example II: Subcutaneous irritation study

1. Test substance:

Test sample: the injection prepared in Example I (with 2.0 g of the active ingredient);

2. Experimental method:

New Zealand rabbits were selected for the experiment, each of which was subcutaneously administrated with the test sample to the left and right sides of each given rabbit body for self-control, and local subcutaneous irritation responses at the administration sites produced by a total of 4 subcutaneous injections were observed. During the experiment, no abnormality related to the injectable pharmaceutical composition of the present invention was found at the local administration sites of all animals.

Experimental Example III Study on therapeutic effect in type 2 diabetic db/db mice after single subcutaneous injection of the pharmaceutical composition of the present application

1. Test substance:

Test sample: the injection prepared in Example I (with 2.0 g of the active ingredient);

2. Experimental method

The effects on blood glucose, serum insulin level, body weight and food intake in the type 2 diabetic db/db mice after single administration were inspected while observing the duration of efficacy after the single administration. The experimental results show that the pharmaceutical composition can obviously lower random blood glucose in the db/db mice after a single subcutaneous injection at a dose of 1, 3, or 10 nmol/kg, and showing a good dose–effect relationship, and showing an obvious random blood glucose lowering effect at a dose of 3 nmol/kg. The duration of the blood glucose lowering effect

of the pharmaceutical composition after the single subcutaneous injection was dose-related, with the random blood glucose lowering effect for a duration of 1 day at a dose of 1 nmol/kg, and for a duration of 2-3 days at doses of 3 nmol/kg and 10 nmol/kg.

Effects of the pharmaceutical composition of the present invention on random blood glucose decrease rate in db/db mice after single administration

Group	Vehicle	Pharmaceutical composition of the present invention		
Dose (nmol/kg)	-	1	3	10
Time	Blood sugar decrease rate (Mean)			
Before administration	-	-	-	-
1 h	5.30	12.27	35.84***	38.65***
2 h	-12.99	6.57	27.78**	39.07***
4 h	5.53	10.00	27.24	43.05***
6 h	-12.54	23.30*	33.84**	43.32***
8 h	10.19	26.40	28.30	51.93***
24 h	-13.98	-2.62	19.21**	26.70***
32 h	-18.47	-8.84	21.55***	36.97***
48 h	4.73	1.89	12.84	23.67
72 h	10.57	-7.02	2.00	9.05
96 h	4.96	-14.42	4.92	-6.30
120 h	-0.19	-22.01	9.46	1.25
144 h	-18.18	-38.41	-3.38	2.58

A difference in blood glucose decrease rate between groups was analyzed using Dunnett's multiple comparisons test in Two-way ANOVA. Compared with the vehicle group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

In addition, Mouse Insulin ELISA Kit was used to determine the serum insulin levels in db/db mice of each group in 2 h after administration. The results show that after the single subcutaneous injection of the pharmaceutical composition in db/db mice at 1, 3, and 10 nmol/kg, the serum insulin levels in the mice in each dose group were significantly increased compared with those in the vehicle group, showing a good dose-effect relationship; and in addition, increase of the serum insulin is also positively correlated with decrease of the blood glucose.

Effects of the pharmaceutical composition of the present invention on serum insulin in db/db mice in 2 h after 4 single administrations

Group	Number of animals	Insulin (μIU/mL)	Increase rate (%)
Vehicle group	16	1,838.86 ± 359.90	0.00
Pharmaceutical composition of the present invention, 1 nmol/kg	16	5,170.54 ± 1,103.22*	181.18
Pharmaceutical composition of the present invention, 3 nmol/kg	16	7,131.29 ± 1,178.14**	287.81
Pharmaceutical composition of the present invention, 10 nmol/kg	16	8,093.32 ± 1,490.42***	340.13

A difference in serum insulin between groups was analyzed using Fisher's LSD test in One-way ANOVA. Compared with the vehicle group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Experimental Example IV: Study on therapeutic effect in type 2 diabetic db/db mice after long-term subcutaneous injection of the pharmaceutical composition of the present application

1. Test substance:

Test sample: the injection prepared in Example I (with 2.0 g of the active ingredient);

2. Experimental method:

Type 2 diabetic db/db mice were administered once every 3 days for 9 consecutive times, and after the end of the experiment (d 26), the liver weights of the db/db mice in the 1, 3, and 10 nmol/kg groups were 2.313 ± 0.113 g, 2.267 ± 0.108 g, and 1.993 ± 0.104 g, respectively, all of which were significantly decreased compared with those in the Vehicle group ($p < 0.05$, $p < 0.05$, $p < 0.001$); and there was no statistical difference in pancreas weight compared with the Vehicle group ($p > 0.05$).

In conclusion, the pharmaceutical composition can dose-dependently reduce body weights and liver weights in type 2 diabetic db/db mice by long-term continuous administrations once every 3 days, with obvious random blood glucose lowering effect at doses of 3 and 10 nmol/kg.

Experimental Example V: Pharmacokinetic study in cynomolgus monkeys after single subcutaneous or intravenous injection of the pharmaceutical composition of the present application

1. Test substance:

the injection prepared in Example 3;

2. Experimental method:

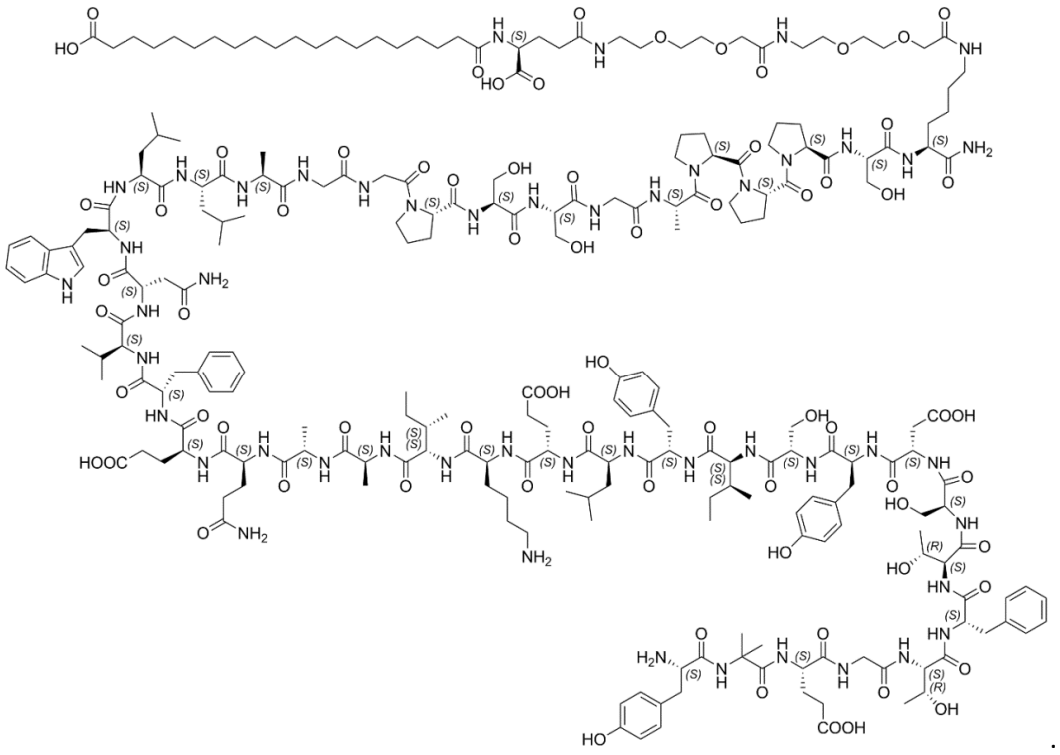
The administration doses for 4 groups, i.e., intravenous injection group, low-dose subcutaneous injection group, medium-dose subcutaneous group and high-dose subcutaneous injection group, were 0.1, 0.03, 0.1, and 0.3 mg/kg, respectively, all by single administration.

After administration by intravenous injection, the drug of the present invention exhibited an elimination half-life t1/2 between 50.7 and 51.5 h in cynomolgus monkeys, indicating slow elimination; a clearance rate (CL) between 0.89 and 0.93 mL/h/kg, indicating low clearance; and an apparent volume of distribution (Vd) between 66.0 and 68.1 mL/kg, indicating that the drug was mainly distributed in plasma. After single administration by subcutaneous injection, the drug of the present invention exhibited a time to peak plasma concentration (Tmax) of 13.3-18.0 h in cynomolgus monkeys; and a plasma elimination half-life T1/2 between 48.9 and 65.4 h, indicating slow elimination; and there was no statistical difference ($P > 0.05$) in the exposure (AUC_{last}) of the drug of the present invention to animals of different genders. In the dose range of 0.02-0.2 mg/kg, the exposure (AUC_{last}) of the pharmaceutical composition in the plasma of the animals was increased with the increase of the dose, and the proportion of the exposure increase was close to that of the dose increase.

Claims

1. A stable pharmaceutical composition of a GIP/GLP-1 dual-receptor agonist, wherein the stable pharmaceutical composition comprises an active ingredient, a buffer solution, an osmotic pressure regulator, and a pH regulator,

wherein the buffer solution comprises a buffer solute and the active ingredient has a structure of:



.

2. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition is an injection, and the concentration of the active ingredient is selected from 0.5 mg/mL to 40 mg/mL, preferably 1 mg/mL to 30 mg/mL.

3. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition comprises 0.05% to 4.0% (W/V) of the active ingredient, preferably 0.1% to 3.0% (W/V).

4. The pharmaceutical composition according to claim 1, wherein the buffer solution is selected from a phosphate buffer solution, an acetate buffer solution, a citrate buffer solution, a carbonate buffer solution, a tartrate buffer solution, a Tris buffer solution, and a histidine salt, preferably a citrate buffer solution or a phosphate buffer solution, and more preferably disodium hydrogen phosphate.

5. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition comprises 0.05% to 3.0% (W/V) of the buffer solute, preferably 0.05% to 2.0% (W/V), and more preferably 0.05% to 1.0% (W/V).

6. The pharmaceutical composition according to claim 1, wherein the osmotic pressure regulator is selected from one or more of mannitol, lactose, sucrose, propylene glycol and glycerol, preferably propylene glycol, or mannitol.

7. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition comprises 0.05% to 5.0% (W/V) of the osmotic pressure regulator, preferably 1.0% to 3.0% (W/V), and more preferably 1.0% to 2.0% (W/V).

8. The pharmaceutical composition according to claim 1, wherein the pH regulator is selected from one or more of hydrochloric acid or sodium hydroxide.

9. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition comprises 0.1% to 3.0% (W/V) of the active ingredient, 0.05% to 3.0% (W/V) of the buffer solute, 0.5% to 5.0% (W/V) of the osmotic pressure regulator, and an appropriate amount of the pH regulator;

preferably the pharmaceutical composition comprises 0.1% to 3.0% (W/V) of the active ingredient, 0.05% to 2.0% (W/V) of the buffer solute, 1.0% to 3.0% (W/V) of the osmotic pressure regulator, and an appropriate amount of the pH regulator;

more preferably the pharmaceutical composition comprises 0.1% to 3.0% (W/V) of the active ingredient, 0.05% to 1.0% (W/V) of the buffer solute, 1.0% to 2.0% (W/V) of the osmotic pressure regulator, and an appropriate amount of the pH regulator; and

further preferably, the pharmaceutical composition comprises 0.1% to 3.0% (W/V) of the active ingredient, 0.05% to 1.0% (W/V) of disodium hydrogen phosphate and/or sodium hydroxide, 1.0% to 2.0% (W/V) of propylene glycol, and an appropriate amount of the pH regulator.

10. The pharmaceutical composition according to claim 1, wherein the weight ratio of the active ingredient to the buffer solute is 1 : 0.01 to 10, preferably 1 : 0.02 to 1, and more preferably 1 : 0.02 to 0.5.

11. The pharmaceutical composition according to claim 1, wherein the weight ratio of the active ingredient to the osmotic pressure regulator is 1 : 0.1 to 20, preferably 1 : 0.4 to 10.

12. The pharmaceutical composition according to claim 9, wherein the buffer solute comprises disodium hydrogen phosphate and sodium hydroxide, and the weight ratio of disodium hydrogen phosphate to sodium hydroxide is 1 : 0 to 3.0, preferably 1 : 0.10 to 2.5, more preferably 1 : 0.13 to 1.5, and further preferably 1 : 0.13 to 1.2.

13. The pharmaceutical composition according to claim 1, wherein the weight ratio of the buffer solute to the osmotic pressure regulator is 1 : 5 to 80, preferably 1 : 5 to 60, more preferably 1 : 15 to 40, and further preferably 1 : 15 to 25.

14. The pharmaceutical composition according to claim 1, wherein the pH range in the pharmaceutical composition is 6.5 to 9.0, preferably 7.0 to 8.5, and more preferably 7.0 to 8.0.

15. The pharmaceutical composition according to claims 1 to 14, wherein the pharmaceutical composition further comprises a preservative selected from one or more of m-cresol, phenol, phenylcarbinol, phenethyl alcohol, parahydroxybenzoate ester, hydroxybenzoate, benzyl alcohol, chlorobutanol, phenoxyethanol and methyl paraben.

16. A method for preparing the pharmaceutical composition according to claims 1 to 15, wherein the method comprises dissolving the buffer solute and the osmotic pressure regulator in water for injection, dissolving the active ingredient in the above-mentioned drug solution, stirring same for dissolution, adding the pH regulator, adjusting same to a constant volume, and filtering and subpackaging same.

17. Use of the pharmaceutical composition according to claims 1 to 15 for the manufacture of a medicament for the treatment of non-insulin-dependent diabetes mellitus, insulin-dependent diabetes mellitus, obesity, insulin resistance, or disorder of blood lipid metabolism, wherein preferably, the non-insulin-dependent diabetes mellitus is type 2 diabetes mellitus.